PCR PURIFICATION PROTOCOL: BD BIOSCIENCES®

This protocol is adapted from a BDBiosciences protocol by the Gene Expression Lab.
This protocol is for use with BD Biosciences' BD Advantage 2 PCR Enzyme System. For additional technical inquiries, contact Technical Service at 877-232-8995 or www.bdbiosciences.com

BEFORE STARTING THE EXPERIMENT
PCR PURIFICATION PROTOCOL
TROUBLESHOOTING

BEFORE STARTING THE EXPERIMENT

Use PCR Hood
For ~30 minutes prior to starting procedure, use the UV light to decontaminate the hood.

Heat Elution Buffer
Make an aliquot of EB in a 1.5ml eppendorf tube and place in incubator at 70°C until ready to elute purified product. Warming the elution buffer may allow for increased yields.

Preparing Plate to collect samples for purification
Take out PCR plate so that the samples can thaw.
Centrifuge plate briefly before collecting samples.

PCR PURIFICATION PROTOCOL

1. After PCR is complete, adjust the reaction volume to 50-100ul with TE (pH7-7.5)
    - Bring the total volume up to 100ul w/ TE; if have over 100ul of total vol., for every 100ul over use increased amts of binding buffer appropriately.
2. Add 4 volumes of Purelink Binding buffer to sample and mix well.
3. Insert the Purelink Spin Column into a 2-ml collection tube. Load 500ul of the sample into the column. Centrifuge at 10,000xg for min at room temperature. COLLECT flow-through.

4. Reinsert the column in the 2ml Collection Tube. Add 650ul of Wash Buffer with ethanol to the column. Centrifuge at 10,000xg for 1 min at room temperature. COLLECT flow-through.
5. Put Purelink Spin Column back in the 2-ml Collection Tube and Centrifuge at 10,000xg for 2 min to remove residual buffer from the membrane filter COLLECT flow-through.
6. Place the Purelink Spin Column in a clean 1.5-ml microcentrifuge tube. Add Appropriate amt of Elution Buffer and incubate at room temperature for 1min.
7. Centrifuge at 10,000xg for 2 min.
8. Determine yields by running DNA 7500 chip.

TROUBLESHOOTING

POOR DNA YIELD

- Reagents may not be prepared properly – make sure that the proper amount of ethanol and isopropanol were added to their corresponding buffers.
- The binding conditions may not have been optimal. For efficient DNA binding, 1 volume of PCR (50-100ul) must always be mixed with 4 volumes of Binding Buffer.